

Citrullination breaks T-cell tolerance in patients with inflammatory arthritides and in healthy persons.

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Objectives:

Rheumatoid Arthritis (RA) and Spondyloarthritis (SpA) are two inflammatory auto-immune diseases. They can be distinguished by their clinical presentations and the presence of antibodies against citrullinated proteins (ACPA), which are specific for RA. Production of these serological markers indicates a predominant role for B-cells in the pathology of RA. However, little is known about the effect of citrullinated proteins on T-cell reactivity.

Methods:

PBMC were isolated from healthy volunteers, RA-patients (CCP+ and CCP-) and SpA-patients. The IFN γ -production was evaluated by ELISpot analysis. PBMC (500000) were stimulated with *in vitro* citrullinated (*cit*) and non-citrullinated (*non-cit*) human cell extract, each at a concentration of 20 μ g/100 μ L. In parallel, 10⁶ PBMC were cultured for a week in the presence of *cit* (80 μ g/ml) or *non-cit* (80 μ g/ml). Supernatants were collected and the secretion of cytokines was evaluated by multi-ELISA detecting IL-2, IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IL-17a, IFN γ , TNF α , G-CSF and TGF β .

Results:

In healthy controls and SpA-patients the number of spots detected by ELISpot differed significantly between stimulation with 'cit' and 'non-cit'. However, this was not seen in RA-patients. These results were confirmed by ELISA. The ELISpot assay showed no significant difference between the number of spots per 500000 PBMCs after stimulation with 'non-cit' or with cDMEM.

Other cytokines, like IL6, IL10, IL17a and G-CSF, did show a distinct increase when PBMC of healthy persons or patients with arthritis were stimulated with 'cit' as opposed to 'non-cit'. On the contrary, a higher production of TGF β was seen in the 'non-cit' condition.

Conclusion:

These data indicate that, unexpectedly, citrulline-specific IFN γ producing T-cells are present in the repertoire of healthy people. However, they seem to be underrepresented in the blood of RA-patients compared to healthy controls and SpA-patients. It is possible that the citrulline-specific T-cells in RA may be retained in the joint, the site of inflammation in RA.

The Multi-ELISA shows distinct cytokine profile of PBMC after stimulation with citrullinated proteins.

Our results indicate a break in T-cell tolerance against citrullinated proteins. The presence of citrulline specific T-cells could play an important role in the ACPA production in RA.